

REMARKS

This application was filed on May 3, 1995, and *ex parte* prosecution has recommenced after an extended delay due to an interference proceeding, in which the Applicants were deemed to have been first to invent the subject matter of a count defined, in part, by the claims that were pending at the time that the outstanding restriction requirement was issued.

I. INTERVIEW SUMMARY

The Applicants thank the Examiner and her supervisor for the courtesy extended to the applicants and their representatives in the interview on August 15, 2006, during which time the restriction requirement, history of the patent family, existence of related applications, and intention to file new claims were discussed.

II. ELECTION WITH TRAVERSE AND RESPONSE TO RESTRICTION REQUIREMENT

In the restriction requirement the Patent Office alleged that the subject matter of claims 41-43, drawn to chimeric genes, were separate and distinct inventions, and required an election. However, the Patent Office acknowledged that claims 3, 5, 39-40, and 44-45 “link” the three allegedly distinct inventions. As such, none of claims 3, 5, 39-40, and 44-45 were divided into specific groups in the restriction requirement.

Because a “complete” reply is required to contain an election, the Applicants hereby elect Group I, drawn to the *B.t. tenebrionus* subject matter of claim 41.

However, the amendments filed herewith have, for practical purposes, rendered moot both the restriction requirement and the election. Specifically, the claim set that is pending as a result of this submission is directed solely to method claims. The new claims are properly classified as “linking claims” (along with the method claims that existed at the time of the restriction requirement and that were never divided between Groups I-III). Because the restriction requirement is moot, it should be withdrawn.¹

¹ Dependent method claim 89 recites use of a *B.t. tenebrionus* coding sequence. Dependent claims 83, 86, and 90 recite use of a *B.t. entomocidus* or *B.t.* P2 sequence.

The MPEP instructs that, even if claims are directed to inventions that are deemed patentably distinct, the Patent Office should not issue a restriction requirement if there would be no serious burden examining the claims to the distinct invention. Because the restricted claims have survived the interference proceeding, it should be clear that they are directed to patentable subject matter, and no serious burden would exist in examining all of the restricted claims. For this reason, the restriction requirement should be lifted.

Additionally, the Patent Office is reminded that the present application is a “pre-GATT” application with an effective filing date more than three years prior to June 8, 1995, so any restriction requirement evaluation, now or in the future, should be subject to the provisions of 37 CFR 1.129, which modify “normal” restriction practice. To the extent that the current requirement is not in compliance with Rule 129, the Applicants traverse.

The Applicants reserve the right to pursue claims directed to the subject matter of any canceled claim in a related application, such as continuing or co-pending applications. Claims have been canceled or amended solely for the purpose of maximizing patent term, and not for reasons relating to patentability.

III. COMPLIANCE WITH THE SEQUENCE RULES

As requested in the restriction requirement, the applicants have filed a new sequence listing herewith (paper and computer readable versions) containing the polynucleotide sequences and an amino acid sequence explicitly set forth in the specification (including the figures). While the specification adequately describes amino acid sequences *encoded by* polynucleotide sequences in the application, the Applicants have concluded that the PTO’s rules do not require their inclusion in the sequence listing.

Many of the amendments to the specification provide cross-references to the sequence listing.

IV. EXPLANATION OF AMENDMENTS

All of the amendments made herein find support in the application as originally filed, and also in the earliest priority application, as briefly explained below.

A. Remarks relating to amendments to the specification.

Most of the amendments to the specification are formal in nature rather than substantive, e.g., updating the status of priority applications; correcting typographical errors; and providing cross-references to the sequence listing. None of these changes introduce “new matter.”

A few of the amendments introduce current nomenclature for *B.t.* genes that were described in the original application. The introduction of current nomenclature does not change the scope or meaning of the application and does not introduce “new matter.” However, the nomenclature may be useful for examination purposes and useful for the interested public reading the eventual patent. The new nomenclature is not used in any pending claims.

Along these same lines, the Applicants wish to provide the examiner with cross-references between various Monsanto constructs referenced in the application by a “pMON” number; the amended sequence listing; and a brief description of the insert contained in the construct. This table, too, may be useful as a cross-reference during reading or examination of the application.

pMON	SEQ ID NO
1982	14
1984	15
5367	6
5370	1
5377	3
5383	5
5390	7
8642	1
8643	3
8644	7
8652	2
9921	2
10506	9
10517	12
10518	12
10526	11
10806	5
10811	5
10814	12
10815	5
10817	5
10819	5

pMON	SEQ ID NO
10821	5
10822	12
10825	12
10827	5
10828	12
10838	12
10839	12
10902	12

B. Remarks relating to the canceled claims.

Claims 13-38 were canceled at the time that the application was filed. In a preliminary amendment entered on November 29, 1995, claims 1, 2, 4, and 6-12 were canceled; claim 5 was amended; and new claims 39-43 were added. In an "Amendment B" filed on May 8, 1997, contingent on the granting of a preliminary motion (Motion 6) in an interference, claims 44-45 were proposed to be added to the application. In an "Amendment C" filed on May 8, 1997, contingent on the granting of a preliminary motion (Motion 11) in an interference, claim 46 was proposed to be added to the application. In an order dated September 4, 2002, Fischhoff's interference Motion 6 was denied and Motion 11 was dismissed. Thus, it appears that claims 44-46 were never formally entered into the application. However, for absolute clarity, those claims are listed herein as canceled (without prejudice). Thus, claims 3, 5, and 39-43 were pending at the time that the restriction requirement was issued. In this paper, all "product" claims have been canceled, and new method claims 47-111 have been added.

The applicants are presently pursuing *method* claims in this application following a lengthy, three-party interference, in which Fischhoff and Perlak were declared to be the first to have invented this invention. The amendments to cancel claims to products are made solely to maximize patent term for the method claims, and are made without prejudice. The Applicants reserve the right to pursue product claims in related applications, such as U.S.S.N. 10/102,469, currently pending before Examiner C.M. Kam. Also pending in this family, and presently before the current examiner, is related U.S. Patent Application Serial No: 11/311,778, with claims directed to plants with two distinct *B.t.* insect tolerance proteins and making/using such plants.

C. Exemplary support for new claims.

The amended claims all find support throughout the application as filed, and the following remarks are merely examples of such support.

1. The original claims provide support for the new claims.

Original claims 1-12 and 27 in the application each are directed to a method, and relate to removal of ATTTA sequences and/or polyadenylation signal sequences from a structural gene sequence.

Notably, original claim 1 was directed to a method and involved reducing the number of polyadenylation signal sequences recited in a Markush group that corresponds to the sequences listed in Table II. (See also claims 6-8.) Dependent claim 2 was further directed to reducing the number of ATTTA sequences. Original claim 3 specified that the gene sequence to be modified be one that encodes an insecticidal protein of *Bacillus thuringiensis*, and specifies that the elimination of problem sequences is performed while retaining a sequence which encodes the protein. Original claim 27 specified removal of sequences comprising more than five consecutive (A+T) or (G+C) bases from the coding sequence. The original claims recite a purpose of improving/enhancing expression in plants.

Thus, the original claims provide support for the new claims presented here, which recite many of the same features or embodiments.

2. The specification provides support for the new claims.

The pending application summarizes a problem that existed with respect to achieving high levels of expression of some foreign genes when introduced into plant cells, and provides solutions invented by Fischhoff and Perlak.

One aspect of the invention is a *method* of making a structural gene, that implements a solution of the inventors, namely, the removal (for example, through site-detected mutagenesis) of “problem sequences”² selected from the sequence ATTTA (equivalent to AUUUA when it appears in RNA) and polyadenylation signal sequences, a list of which are provided in Table II (p. 36), and other locations in the application, and in the

² The term “problem sequences” is used herein colloquially to refer to ATTTA or polyadenylation signal sequences, and is not used in the claims or intended as a term of art.

original claims. Embodiments involving mutagenesis find support throughout the application, including at p. 22, lines 27-30; p. 25, lines 1-28; and the examples.³ A related solution of the inventors involves the use of an intended amino acid sequence (optionally deduced from wild type coding sequences) for *de novo* synthesis of a structural gene sequence, where the synthetic sequence has reduced numbers of problem sequences relative to the wild type gene sequences from which the intended amino acid sequence was previously derived. See, e.g., p. 23, lines 19-21; and p. 28, lines 25-28. (See new claims 59-66 and 111, for example.)

Support for claiming a method for making a structural gene is found, e.g., at page 14, first paragraph; and page 16, lines 15, to page 17, line 9. A focus of the current claims is directed to making structural genes that encode a polypeptide with insecticidal activity; and support for such claim recitations is found in these same passages and repeatedly throughout the application.

The invention is generally applicable for making new structural genes for any protein,⁴ and it is especially applicable for making structural genes for proteins derived from *Bacillus*, because bacteria of the genus *Bacillus* have genomes that are unusually rich in adenines and thymidines [(A+T)-rich]. (The specified problem sequences are themselves (A+T)-rich, and are apt to occur with greater frequency in an (A+T)-rich genome.) Support for claim recitations specifying insecticidal proteins derived from *Bacillus* may be found, e.g., at page 16, line 15, to page 17, line 9; and at page 21, line 1, to p. 22, line 12, which explain that the invention is particularly applicable to genes of *Bacillus*, which have among the most (A+T)-rich genomes and, consequently, a greater propensity for (A+T)-rich problem sequences.

Support for claims specifying insecticidal fragments and/or hybrid proteins (or for modifying *portions* of an insecticidal gene sequence) is found throughout the application, especially the examples, which are summarized briefly here.

Example 1 describes a variety of structural gene embodiments generated through site-directed mutagenesis. In some embodiments, approximately the amino-terminal

³ These excerpts provide support for, e.g., claim 110.

⁴ See, e.g., p. 17, lines 5-10 (“[I]t should be understood that the present method may be used to prepare synthetic plant genes which encode non-plant proteins other than the crystal protein toxin of *B.t.* as well as plant proteins”)

one-third of a wildtype B.t.k. HD-1 gene (residues 29-607) was altered to reduce the number of problem (ATTTA or Table II polyadenylation signal) sequences. Site-directed mutagenesis reduced the number of polyadenylation sequences from 18 to 7 and the number of ATTTA sequences from 13 to 7, achieving modified sequences that exhibited improved expression in plants.

In still another embodiment, the inventors demonstrated that removal of as few as three polyadenylation signal sequences in the “240 region” of the gene⁵ was result-effective.

In still other variations, within the amino-terminal one third of the gene, the inventors made alterations to approximately the first third (bases 1-590) or the second two thirds (bases 590-1845). Both constructs were expressed and resulted in insect toxicity.

Example 2 describes an experiment in which a synthetic insecticidal fragment (amino acids 1-615) of Btk HD-1 was made that was devoid of ATTTA sequences and substantially devoid of Table II polyadenylation signal sequences (in this instance, only one).

Example 3 describes experiments in which genes encoding chimeric insecticidal proteins were constructed using sequences derived from *Bacillus*. For example, the inventors took the 5' two-thirds of a synthetic HD-1 gene and combined it with the 3' one-third of an HD-73 sequence, modified via site-directed mutagenesis. The resulting construct was devoid of ATTTA, and substantially devoid (reduced in this example from 18 to 2) of Table II polyadenylation signal sequences, and it encoded an *insecticidal hybrid protein* comprised of a fusion of fragments of more than one insecticidal protein derived from *Bacillus*. Still other embodiments in Example 3 include sequence alterations (e.g., Met-Ala at the beginning of the coding sequence), partly modified genes, and fully synthetic genes.

Example 4 describes numerous experiments that showed that genes partly modified to reduce the number of problem sequences (ATTTA or Table II polyadenylation signal sequences) were more highly expressed than wild type genes, and that fully synthetic genes were still more highly expressed; and that insect resistance conferred to transformed plant cells was correspondingly increased in a variety of commercially significant crops.

Still further embodiments involving removal of the same types of problem sequences from other *Bacillus* genes are described in the later examples.

⁵ So nicknamed based on the primer (BTK240) used to make this construct.

Page 70 describes knowledge that fragments of *B.t.* toxins are fully insecticidal. (See also p. 90, lines 1-8.)

One or more claims include a clause specifying, e.g., starting with one or more coding sequences derived from *Bacillus* that encode insecticidal polypeptide(s) and that comprise a plurality of occurrences of ATTTA sequences or polyadenylation signal sequences listed in Table II. (See, e.g., claims 47-58.) This clause provides antecedent basis for a subsequent, relativistic statement in the claim specifying, e.g., making a structural gene that comprises a coding sequence that *contains fewer* occurrences of the ATTTA sequence(s) or *fewer* of the polyadenylation signal sequences compared to the *Bacillus*-derived sequences.”⁶

Claim phrases relating to making a structural gene find support throughout the application, which teaches site-directed mutagenesis procedures, *de novo* synthesis procedures, and combinations thereof performed on different sequences that are combined to make a structural gene. See, e.g., pp. 21-23 and the Examples. Claim 47 and other claims specify starting with a coding (nucleotide) sequence, from which a structural gene can be made by any of these techniques. In contrast, claims 59-66 specify starting with an amino acid sequence, from which a structural gene can be synthesized *de novo* using the sense codons shown, e.g., in Table I, optionally without reference to the original *Bacillus* nucleotide sequences from which the amino acid sequence may have been derived. (See, e.g., p. 28, lines 23-28: “It is evident to those skilled in the art that while the above description is directed toward the modification of the DNA sequences of wild-type genes, the present method can be used to construct a completely synthetic gene for a given amino acid sequence.”)

Claim 47 and other claims include a step specifying, e.g., “reducing the number of said ATTTA sequences or the number of said polyadenylation signal sequences in the coding sequence [derived from *Bacillus*] by substituting sense codons for codons in the coding sequence.” A step of substituting codons finds support throughout the application,

⁶ This requirement that the resultant structural gene contain fewer of the problem sequences, compared to the wild type sequences from which the polypeptide was originally derived, applies to methods of making the full length, hybrid, and fragment embodiments of the invention, and serves to distinguish any prior art in which problem sequences may have been fortuitously deleted through gene truncation to create a truncated structural gene encoding an insecticidal fragment, but where no changes were made to remove problem sequences from the truncated sequence itself.

including pp. 25-28 and numerous portions of the application relating to site directed mutagenesis procedures for altering codons that contribute to problem sequences. The term “sense” codons finds support, e.g., in Table I, which lists only the 61 “sense” codons (the codons that encode amino acids) and not the three “nonsense” (stop) codons. Thus, reducing the number of problem sequences by substituting sense codons refers to a reduction through alteration of the coding sequence, as distinct from a reduction achieved solely through shortening the coding sequence by introduction of a stop codon (truncation).⁷ It will be appreciated that a codon substitution can be achieved by changing one, two, or all three nucleobases of a codon.

New claim 55 recites, in step (a), “starting with coding sequences, from one or more structural genes derived from *Bacillus*” Subsequent steps relate to reducing the number of problem sequences in the coding sequences by substituting sense codons; and making a making a structural gene that comprises the coding sequences with the substituted codons. Support for starting with coding sequences from more than one gene is found in numerous examples, as described above, in which hybrid or chimeric genes were constructed. (See also discussion above of hybrid proteins, applicable to claims 49, 51, and 64, for example).

Claim 59 and other claims make reference to “wild type” *Bacillus* sequences. The term “wild type” (or “wild-type”) is used throughout the application, especially the examples, to refer to structural genes isolated from an organism e.g., *Bacillus*. Likewise, the teachings that the problem sequences are present in the wild type sequences and their numbers should be reduced or substantially eliminated is found throughout the application, including at page 22, line 24, to p. 23, line 13.

Some claims recite removal of ATTTA *or* polyadenylation signal sequences whereas others specify removal of both. These variations find support throughout the application, including at page 22, line 24, to p. 23, line 13. (“It is most preferred that substantially all the polyadenylation signals and ATTTA sequences are removed although enhanced expression levels are observed with only partial removal of either of the above identified sequences.”) Removal of substantially all of a problem sequence renders the resulting coding sequence “substantially devoid” of the problem sequence (a term used, e.g., in original claim 13 and new claims 63-68).

⁷ See note 6, above.

Claims 69-111 in the new claim set are multiply dependent and specify further details relative to one or more of claims 47-68.

For example, claims 72 and 76 recite that the claimed structural gene contains no more than one occurrence of the sequence ATTTA or/and no more than one occurrence of the polyadenylation signal sequences listed in Table II. These embodiments find support, e.g., in Figure 1; at page 23, line 28, to p. 24, line 2; and in Example 2 (p. 50, lines 25-27).

The recitations in claims 71 and 74 of no more than seven polyadenylation sequences and no more than seven ATTTA sequences find support, e.g., at p. 39, lines 27-31, and p. 42, lines 8-10.

The recitation in claim 75 of no more than two polyadenylation sequences finds support, e.g., in Example 3 at p. 53, lines 15-17.

Claim 78 specifies making a structural gene with a (G+C) content of about 50%. This limitation finds support, e.g., at p. 29, lines 3-5.

Claims 79-80 specify starting with a coding sequence with an (A+T) content of about 62%. This limitation finds support, e.g., at p. 21, first paragraph.

Claims 81, 84, and 87 specify a sequence derived from *Bacillus thuringiensis* (*B.t.*). Claims 82, 85, and 88 specify a *B.t.* crystal protein sequence. These embodiments find support throughout the application, including at p. 14, lines 10-11; p. 16, line 25, to p. 17, line 2; and in the examples.

Claims 83, 86, and 90 specify a *B.t.* P2 sequence or a *B.t.* entomocidus gene sequence. These embodiments find support throughout the application, including in Examples 6 and 7; Figure 13; and p. 15, line 29, to p. 16, line 7.

Claim 89 specifies use of a *B.t. tenebrionus* sequence, which finds support in Example 5 and elsewhere in the application.

Support for claims 97-99, which relate to removing the problem sequences while retaining the encoded amino acid sequence, is found, e.g., at p. 25, lines 4-6, and original claim 3.

The application teaches to avoid introduction of codons that are rarely found in plant genomes at, e.g., p. 23, lines 13-16. (See, e.g., new claim 100.)

Claims 101-102, relating to avoidance of certain nucleotide doublets, finds support, e.g., at p. 25, lines 22-24; p. 28, lines 28-30; and p. 39, lines 17-18.

Claim 103-105 specify codon modification to reduce the number of regions with greater than five consecutive adenine (A) or thymine (T) nucleotides. This variation is described throughout the application, including in Figure 1; at p. 24, lines 19-29; at p. 25, lines 20-22; and at p. 28, lines 28-29; and in an original claim, as noted above.

Examples 1-3 provide support for an embodiment involving a truncated structural gene, as recited in claim 106.

Claim 107 specifies attaching a plant promoter to the structural gene. This embodiment finds support, e.g., at p. 29, line 22, to p. 31, line 24.

Claim 108 specifies including in the structural gene a sequence that encodes an amino-terminal chloroplast transit peptide or a secretory signal sequence. This element finds support, e.g., at p. 32, lines 1-5, of the application.

Claims 109 specifies attaching to the structural gene a 3' non-translated nucleotide sequence that comprises a plant polyadenylation signal. This element finds support, e.g., at p. 29, lines 18-22; and at p. 32, lines 6-19 of the specification.

V. CONCLUSION

The Patent Office is authorized to charge any fee deficiencies necessary for entry of this submission, and other fees that may arise in this case (other than the issue fee) to deposit account No. 13-2855, under order number 28079/41785.

For the foregoing reasons, the restriction requirement should not be maintained. The applicants request prompt examination of all of the pending claims, which are believed to be in condition for allowance.

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